

Environmental Factors and Diseases of the Pancreas

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The five major diseases of the pancreas together make a significant contribution to morbidity and mortality among the people of the United States. These diseases are diabetes, cystic fibrosis, acute and chronic pancreatitis, and carcinoma of the exocrine pancreas. Four of these diseases can be modeled in laboratory animals by acute or chronic administration of chemical poisons or carcinogens. Human pancreatic diseases attributed to the effect of chemical agents including alcohol and drugs include many cases of chronic pancreatitis and some cases of acute pancreatitis. The cause is not known in many cases of human pancreatitis, including interstitial, acute, and chronic clinical forms. Epidemiologic studies suggest that the increasing incidence of carcinoma of the exocrine pancreas in the United States may reflect chemical carcinogenesis. On the basis of experimental observations, we know that pancreatic islet cells can be damaged directly by toxic chemicals, and that islet cell tumors can be chemically induced.

Thus, there is adequate background data to conclude that several pancreatic diseases of obscure etiology may be due in part to hitherto unidentified toxic effects of chemical agents encountered in personal or general environments.

The aggregate impact of five major diseases of the pancreas on mortality and morbidity in the United States is major. These diseases are diabetes, cystic fibrosis, acute and chronic pancreatitis, and carcinoma of the exocrine pancreas. The last of these is clearly increasing in incidence and environmental influences are suspected of playing an etiologic role. In an international survey of pancreatic diseases observed at autopsy, nearly two people out of every 100 had apparently died from a pancreatic disease (1), and the prevalence of diabetes as an underlying cause of disease has been stressed (2).

Homeostasis and Disease in Humans

The pancreas, which constitutes about 0.1% of adult body weight, is highly specialized to synthesize and secrete several specific proteins. More than 90% of the gland consists of exocrine pancreatic acinar cells which are as active in protein synthesis as any cell type in the body, secreting digestive enzymes into the intestine through the pancreatic duct system. These hydrolytic enzymes, when

activated within the pancreas, can digest protein, RNA, DNA, phospholipids, carbohydrates, and lipids at physiologic pH. This creates a special vulnerability of pancreatic acinar cells to injury which can trigger activation and release of these enzymes within the cell, resulting in cytoplasmic damage or cell death. Minor degrees of injury seem to occur regularly, resulting in autophagy and recovery of the injured cell, or necrosis and loss of individual acinar cells. The causes of acinar cell damage include ischemia, trauma, duct obstruction, nutritional deficiency, metabolic abnormalities, virus infection, and toxic chemicals. Acinar cells can divide in experimental animals, and we assume that regeneration can occur in the human. Initial sections of this paper focus on diseases of exocrine pancreas, and a final section focuses on the pancreatic islets.

Pancreatitis

When many acinar cells are injured and their enzymes are released, virtually all of the gland may be destroyed. The resulting clinical disease, acute hemorrhagic pancreatitis, may be fatal. Less severe degrees of the process may destroy part of the gland and lead to scarring or pseudocyst formation. Multiple minor episodes may cause the majority of the gland to be replaced by fibrous tissue so that en-

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zyme secretory function is lost leading to defective digestive function in the small intestine. Such scarred, atrophic glands are characteristic of chronic pancreatitis. Milder degrees of acute pancreatitis in which complete recovery is the rule also occur. This disease is usually designated as interstitial pancreatitis.

Acute pancreatitis is a significant clinical problem because of associated mortality and morbidity including the possible late complications indicated above. The mortality rate among "first episodes" of acute pancreatitis was 20% in a recent British study (3).

Many cases of acute pancreatitis are associated with biliary tract disease, which may lead to obstruction of the pancreatic duct by gallstones when the two duct systems join in the head of the pancreas. Trapnell (3) tabulated the etiologic factors among 590 cases of acute pancreatitis (Table 1).

Table 1. Etiological factors in pancreatitis.

Etiological factor	No. cases	%
Biliary tract disease	316	53.6
Chronic alcoholism	26	4.4
Mumps	7	1.2
Hyperparathyroidism	1	0.2
Carcinoma	8	1.4
Steroids	6	1.0
Other	9	1.5
Not stated ^a	14	2.4
Idiopathic	203	34.4
TOTAL	590	100

^aIn these cases the presence or absence of biliary tract disease had not been determined.

In this country, the proportion of cases associated with biliary tract disease has been reported as lower (37%), and that due to alcoholism as higher (19%) (4). The incidence of episodes of acute pancreatitis is high among chronic alcoholics. Fully one third of cases of acute pancreatitis have no apparent cause, and this so called "idiopathic" group probably reflects several different causes. Pancreatitis, like cirrhosis, is correctly regarded as several diseases in regard to etiology.

There was no convincing evidence of increasing incidence of acute pancreatitis in the period of 1950-1969 in Trapnell's study, nor during 1940-1969 in the similar, but smaller series from Minnesota (4).

In this country, the majority of cases of chronic pancreatitis, specifically the variant designated as chronic calcifying pancreatitis, in which a specific cause is known, result from chronic alcoholism. According to the view of Henri Sarles, this reflects multifocal obstruction of the pancreatic ducts

throughout the gland, and his detailed analysis of the effects of alcohol on pancreatic secretion is available in multiple reports and reviews (5, 6). His views are not uncontested (7), but the relationship between chronic alcoholism and chronic calcifying pancreatitis seems well enough established so that the incidence of the latter will reliably be proportional to the former. Sarles defined chronic alcoholism as a history of drinking a mean quantity of 150 ml of alcohol per day for at least two years. Trapnell reports a rising proportion of cases of acute pancreatitis among alcoholics in his study spanning 1950-1969 (3).

Although many cases of chronic pancreatitis may be chronic calcifying pancreatitis due to chronic alcoholism it is usually accepted that some cases may represent the end stage of damage accumulated during multiple, recurrent episodes of nonfatal pancreatitis due to causes other than alcoholism. A recent Japanese study indicates that etiology was unknown in 41.5% of 150 cases of chronic calcifying pancreatitis, and an additional 11.3% had a history of previous acute pancreatitis but were not alcoholics (8). It follows that any toxic chemical capable of inducing pancreatitis with necrosis and scarring could cause chronic pancreatitis if there were multiple episodes of exposure and pancreatic injury.

The exocrine pancreas appears to possess great functional reserve. In patients with chronic pancreatitis, it appears that atrophy and scarring must progress to the point that enzyme secretion is reduced below 10% of normal levels before malabsorption occurs and fecal fat and nitrogenous compounds increase (9).

Cystic Fibrosis

Cystic fibrosis is a genetically determined and inherited systemic disease in which the pancreatic changes may be characterized as a variant of chronic pancreatitis induced by obstruction of intrapancreatic ducts by abnormal mucinous secretions. This process begins *in utero* and progresses to complete destruction and atrophy of the exocrine pancreas during the first few years of life. The gene for cystic fibrosis is relatively common in the general population; estimated to be about 1/20 among Caucasians (10). This frequency has been interpreted as suggesting that there may be some selective advantage for heterozygotes (11). Another possibility is that this is a common mutation which is continually being introduced into germ cells in the population. While the conventional view is that germ cells are remote and well protected from environmental mutagens, the possibility of inducing

germ cell mutations which result in genetic disease is real.

We do not have evidence that the incidence of cystic fibrosis is rising in this country. Inability to reliably recognize cystic fibrosis heterozygotes in the general population has prevented assessment of whether they are at high risk for development of pancreatic or pulmonary disease under the influence of environmental factors.

Anomalies and Aging

Developmental abnormalities including agenesis of the exocrine pancreas and selective trypsinogen deficiency have been described (12). Such rare events could reflect teratogenic effects of external agents, but no constant etiologic relationships have been defined and the occurrence of such disorders is sporadic. Although autopsy of aged individuals sometimes reveals an apparent decrease in mass of the exocrine pancreas, loss of endocrine function with age seems to be a more significant consideration than loss of exocrine function.

Carcinoma of the Pancreas

Carcinoma of the pancreas is an increasingly prevalent form of human cancer (13, 14). Several epidemiologic studies suggest that chemical carcinogens may induce pancreatic cancer in humans, and several chemicals have been demonstrated to induce carcinoma of the exocrine pancreas in experimental animals following systemic administration.

Bates has recently reviewed background considerations relevant to chemical carcinogenesis in the pancreas, and cites studies showing an increased incidence of pancreatic cancer among workers occupationally exposed to β -naphthylamine or benzidine, among chemists who were members of the American Chemical Society (15) and among cigarette smokers (16). Epidemiologic data indicate that although the incidence of carcinoma of the pancreas in Japan is low compared with that in the USA, Japanese who have immigrated to the US have a higher incidence than that of the native white population of this country. Thus, environmental factors appear to play a major role in the etiology of carcinoma of the pancreas.

Experimental Toxicology and Pancreatic Damage

Numerous chemical agents are known or suspected to induce acinar cell damage or necrosis, to alter acinar cell or duct cell function, or to induce

neoplastic change in the pancreas. These agents are listed in Tables 2-5. A few generalizations and summary comments are included here and will draw on specific examples cited there.

Varying degrees of acute pancreatitis can be induced in animals by giving chemicals which are cytotoxic for acinar cells. Some systems closely mimic the most severe forms of acute hemorrhagic pancreatitis, e.g. ethionine administration in mice fed a choline-deficient diet (31). Intermediate degrees of pancreatic necrosis simulating nonfatal degrees of pancreatitis have been induced by puromycin, azaserine, and hydroxyaminoquinoline 1-oxide, whereas lower doses of these and other agents, or treatment with less cytotoxic agents may induce milder degrees of damage analogous to human interstitial pancreatitis. The list of agents affecting the pancreas includes several amino acid analogs. This group of compounds has a specific propensity to affect the pancreas, probably because of the pancreas' high affinity for α -amino acids. The pancreas has been shown to concentrate both normal and several abnormal amino acids or amino acid derivatives to a greater degree than most other tissues. Alcohol, and perhaps cortisone, seem to have minimal direct cytotoxic effects on the pancreas, but have been shown to alter pancreatic secretions in a way which promotes protein precipitation in the duct system leading to pancreatitis due to duct obstruction.

Since about a third of cases of human acute pancreatitis are classed as idiopathic, it seems reasonable to suspect that some of these cases may reflect acute toxic effects of chemicals, food additives, or drugs. Medical literature contains sporadic reports of acute pancreatitis in association with specific therapeutic products (Table 3). More detailed analysis of possible occupational or environmental exposure to toxic agents in patients with pancreatitis might lead to the identification of specific causes in the "idiopathic group" of patients with acute pancreatitis, and in non-alcoholic patients with chronic pancreatitis.

Experimental models of induction of pancreatic adenocarcinoma have been described using di-propylnitrosamine derivatives, methylnitrosourea, 4-hydroxyaminoquinoline 1-oxide, and azaserine (Table 4). With this background, environmental nitrosamines should receive special scrutiny as possible pancreatic carcinogens.

Pancreatic Islets

The pancreatic islets of Langerhans are small nodules of endocrine tissue dispersed throughout the pancreas. They number an estimated 0.25-1.75

Table 2. Agents reported to cause cytotoxic effects in exocrine pancreas of experimental animals.

Agent	Effect ^a	Species	Reference
2-Acetylaminofluorene	xRER	rat	Flaks (17)
4-Acetylaminofluorene	xRER	rat	Flaks (18)
Actinomycin D	xRER, AV, deg	mouse	Rodriguez (19)
Aflatoxin	AV, xRER, deg	rat	Rao (20)
Azaserine	xRER, AV, atr, nec	rat	Hruban (21)
Carbon tetrachloride	nec, atr	rat	Veghelyi (22)
Chloroquine	AV	rat	Fedorko (23)
Chlorothiazide	pancreatitis	mouse	Cornish (24)
Cobalt chloride	xRER, AV	guinea pig	Kern (25)
Cortisone	AC atr, fat nec, SENZ	rabbit	Stumpf (26)
Cycloleucine	AV, xRER, deg	rat	Chenard (27)
Diethanolamine	xRER, deg	rat	Hruban (28)
Ethanol	duct obstruction xmito	rat	Sarles (29)
Ethionine	AC, AV, atr, deg, nec, xRER, xmito	rat mouse guinea pig hamster	Farber (30) Lombardi (31) Wenk (32) Boquist (33) Goldberg (34)
5-Fluorouracil	AV, xRER, xmito	rat	Martin (35)
β -3-Furyl-DL-alanine	xRER, AV, xmito	rat	Hruban (36)
4-Hydroxyaminoquinoline-1-oxide	AC nec, SENZ	guinea pig rat	Reddy (37) Konishi (38)
Methionine	AV, fibrillar bodies, atr, deg, xRER	hamster rat	Boquist (39) Kaufman (40)
Neutral red	AV, xRER	mouse	Morgan (41) Weiss (42)
Puromycin	AC nec, xRER, AV	rat	Longnecker (43, 44)
β -3-Thienyl-DL-alanine	AV, xRER	rat	Hruban (45)
Triparanol	AV, xRER, deg	rat	Hruban (28)
Vinblastine	AV, AC atr	mouse	Nevalainen (46)

^aThe observed effect is abbreviated as follows: AC = acinar cell; DC = ductal cell; IC = islet cell; nec = necrosis; atr = atrophy; AV = autophagy; xRER = change in granular endoplasmic reticulum; xmito = mitochondrial change; SENZ = elevation of serum enzymes from pancreas, e.g., amylase; deg = loss of secretory granules.

Table 3. Agents and drugs reported to cause pancreatitis in humans.

Agent	Effect ^a	No. cases	Reference
Azathioprine	AP	1	Breuer (47)
Chlorthalidone	AP op	1	Jones (48)
Chlorothiazide	AP, Lab Dx	4	Johnston (49)
Furosemide (Lasix)	AP, RP, Lab Dx	2	Jones (50)
			Wilson (51)
Immuran	AP, RP, Lab Dx	1	Nogueira (52)
Oral contraceptives	AP, Lab Dx	4	Davidoff (53)

^aThe observed effect is abbreviated as follows: AP = acute pancreatitis; IP = acute interstitial pancreatitis; CP = chronic pancreatitis; RP = recurrent episode(s) of acute pancreatitis; Lab Dx = diagnosis based on laboratory studies; op = operative diagnosis.

Table 4. Agents reported to cause pancreatic neoplasms in experimental animals.

Agent	Effect ^a	Species	Reference
Azaserine	AC ca	rat	Longnecker (54)
2, 2'-Dihydroxydi-N-propylnitrosamine	DC, AC ca	hamster	Pour (55)
N,N'-2-7-Fluorenylene-bisacetamine	AC ad	rat	Morris (56)
Heliotrine	IC ad	rat	Schoental (57)
4-Hydroxyaminoquinoline 1-oxide	AC ad	rat	Hayashi (58)
	AC ca	rat	Konishi (59)
N-Methyl-N-nitrosourea	DC, AC ca	guinea pig	Reddy (60)
Streptozotocin	IC ad	rat	Rakieten (61)

^aThe observed tumor is abbreviated as follows: AC = acinar cell; DC = ductal cell; IC = islet cell; ad = adenoma; ca = carcinoma.

$\times 10^6$ in the adult human and comprise about 1% of the pancreas (62). In aggregate they function as an endocrine organ regulating blood glucose levels and cell metabolism. Islets consist of three major cell types: β -cells make and secrete insulin in response to rising plasma glucose levels, α -cells (sometimes designated α_2 -cells) make and secrete glucagon, and δ -cells (sometimes designated α_1 -cells) make and secrete gastrin.

Islet cells are subject to damage by viral infections and toxic chemicals, and may respond by reversible degenerative changes or cell death. Islets may also be destroyed incidental to diseases of the exocrine pancreas because of the intimate association of the two types of tissue. The prevalence of decreasing β -cell function with age suggests that islets have a limited capacity to regenerate.

Diabetes is the major clinical syndrome reflecting decreased islet cell function and more specifically reflects deficiency of insulin secretion by β -cells. Reduced secretion may reflect a decrease in the number of β -cells or a decrease in insulin synthesis and secretion by β -cells. Several possible causes are recognized for such decrease of β -cell function in humans: an inherited genetic basis and destruction incidental to diseases of the exocrine pancreas. While the most prevalent type of diabetes is regarded as a familial disease, the inheritance pattern is complex, suggesting that several genes may be involved (63). In addition, physiologic, nutritional, and possibly environmental factors seem to influence the onset of the disease, e.g., obesity and age.

Experimental toxicology has demonstrated that β -cell destruction or dysfunction can be caused by chemicals, e.g., alloxan, streptozotocin, cyproheptadine, and others (see Table 5). These observations suggest that normal or genetically predisposed individuals might lose β -cell function as a result of environmental influences such as exposure to toxic chemicals introduced into the environment or used as drugs or food additives.

The incidence of diabetes increases with age, and it may be that "aging" of islet cells is an important factor in the development of adult onset diabetes. Aging theory recognizes the accumulation of somatic cell damage, e.g. somatic cell mutation, as a possible basis for declining function. There is increasing evidence that chemical mutagens can contribute to such change in mammalian somatic cells.

The recent report of the National Commission on Diabetes (71) should be consulted for an exhaustive discussion of etiologic considerations and research needs for this major disease.

There are no well-defined clinical syndromes related to decrease of α - or β -cell function. It is known that α -cells may be damaged toxically by

chemicals such as cobalt. Thus while β -cell damage or dysfunction seems to constitute the major problem attributable to loss of islet cell function, it is possible that other clinical problems will emerge or be defined in the future.

Table 5. Agents reported to be cytotoxic for islet cells.

Agent	Effect ^a	Species	Reference
Alloxan	β nec	several	Dunn (64)
Cobalt chloride	α deg	several	Fischer (65) ^b
Cyclizine	β , deg, xRER	rat	Hruban (66)
Cyproheptadine	β vac, deg	rat	Wold (67)
Streptozotocin	β nec	several	Rakieten (68)
N-Methyl-N-nitrosourea	nec (α , β , δ)	hamster	Wilander (69) Berne (70)

^aAbbreviations used are: β = β cell; α = α cell; δ = δ cell; nec = necrosis; deg = degeneration; xRER = change in granular endoplasmic reticulum.

^bThis recent review provides an analytic discussion of the effect of most of the agents listed in this table.

Islet cell tumors constitute a second group of lesions causing human disease. This may be associated with hypersecretion of insulin, glucagon, or gastrin leading to clinical syndromes attributable to hormone excess, e.g., hypoglycemia, diabetes, and secretory disturbances in the gastrointestinal tract reflected as intractable peptic ulcers or diarrhea. The causes of islet cell tumors remain as obscure as the problem of etiology of cancer in general. Islet cell tumors can be induced in experimental animals by streptozotocin and heliotrine (Table 4). Thus it is possible for chemical carcinogens to affect islet cells under appropriate circumstances.

Detection of islet cell tumors in humans depends on the biologic activity of the tumor. Hormone-secreting tumors may be recognized during life because of clinical evidence of disturbed endocrine function; however, nonfunctioning tumors may be recognized only as a result of metastatic spread or be found incidentally at autopsy.

Recommendations

The preceding considerations suggest several approaches which can be taken to further define the causes of pancreatic diseases and possibly prevent introduction of environmental hazards which might further increase the incidence of these diseases.

Toxicologic evaluation of new chemicals to be utilized in ways which will result in significant human exposure should include evaluation of acute and chronic effects on the pancreas in regard to the

following: (1) acinar cell cytotoxicity; (2) islet cell cytotoxicity; (3) effects on the duct system or pancreatic secretions which will lead to duct obstruction; and (4) cancer induction. Evaluation should include acute and chronic toxicologic studies for all compounds and carcinogenesis bioassay for potential carcinogens. Since so many α -amino acid analogs have been shown to affect the pancreas, such compounds should be especially suspect in regard to effects on acinar cells. Agents which are structural analogs of glucose are particularly suspect for potential endocrine effects because of the likelihood that they may enter or bind to β -cells via glucose receptor pathways.

More sensitive and specific methods are needed to detect "subclinical" degrees of acute acinar cell injury and focal duct obstruction. Population groups at risk from exposures to known pancreatotoxic agents should be sought. Determination of urine and/or serum amylase levels seems to offer the most practical initial screening approach at the present time although this technique lacks specificity for the pancreas. Fundamental research relating to the pancreas should receive increased emphasis and should be focused on the basic mechanisms of toxic injury to pancreatic cells using models of chemical injury to pancreases of animals on a laboratory basis.

If situations arise in which chronic exposure to a suspected toxin has occurred and there is a question regarding exocrine pancreatic damage, the population at risk should be screened for adequacy of pancreatic function. Determination of fecal fat would seem to offer one practical initial screening approach, although more sensitive methods to detect decreased exocrine function should be sought.

Populations considered to be at risk for exposure to any toxic agents suspected of affecting islets should be screened or monitored by tests for glycosuria and serum glucose determinations (fasting or 2 hr postprandial); and further evaluated by glucose tolerance testing if abnormalities are found.

Data regarding the incidence of major pancreatic diseases in defined population groups should be collected and analyzed. If there is evidence of increase in the incidence of any disease, the change should be analyzed to see if there is evidence of an etiologic role for any specific environmental agent or factor.

It remains to be learned whether individuals who are heterozygous for the gene for cystic fibrosis are at special risk for the development of acute or chronic pancreatitis. If this were true, then environmental factors not affecting the general population might carry a special risk for this group. Therefore, development of a reliable test for cystic

fibrosis heterozygotes is needed, since about 5% of the population could be affected (72).

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